Reference: *Biol. Bull.* 171: 360–370. (October, 1986)

A COMPARATIVE STUDY OF MELANIN-CONCENTRATING HORMONE (MCH) ACTION ON TELEOST MELANOPHORES

MAKOTO NAGAI, NORIKO OSHIMA, AND RYOZO FUJII*

Department of Biology, Faculty of Science, Toho University, Miyama, Funabashi, Chiba 274, Japan

Abstract

The action of melanin-concentrating hormone (MCH) on melanophores was studied in 27 teleost species. MCH caused melanosome aggregation in all teleosts studied, including two siluroid catfish in which melanin-aggregating nerves are known to be cholinergic. In most fish, the minimal effective concentration of MCH was estimated to be 10^{-10} *M*, while in three swellfish examined it was higher than 10^{-8} *M*. The mode of action of the peptide was identical in either adrenergically or cholinergically innervated melanophores. It may act through specific receptors on the melanophore membrane. These results suggest that MCH may be a biologically active hormone common to teleosts.

Introduction

Enami (1955) observed pigment aggregation in catfish (*Parasilurus asotus*) melanophores after injecting crude extracts of the pituitary of the same species. He termed the effective principle "melanophore-concentrating hormone" or "MCH," and further indicated that the substance was neither a catecholamine nor an acetylcholine. Imai (1958) confirmed these results. Using the rainbow trout, *Salmo gairdneri,* Baker and Rance (1983) reported that "melanin-concentrating hormone" is a peptide with a molecular weight of less than 2000, and that most of its bioactivity occurs in the hypothalamus, favoring the hypothalamic origin of the hormone theory, which had originally been surmised by Enami (1955). Using immunohistochemical electron microscopy, the results of Naito *et al.* (1985) supported these findings.

Meanwhile, Kawauchi and his colleagues (1983) determined the primary structure of MCH peptide purified from chum salmon (*Oncorhynchus keta*) pituitary. Unrelated to any known hormonal peptide, it was found to be a novel hormone, consisting of 17 amino acids and a sulfhydryl link. Subsequently, the same molecular species of MCH was synthesized and its action to aggregate melanosomes (melanin-bearing dark organelles within melanophores) was confirmed in a few teleosts by Wilkes *et al.* (1984). The mechanism of MCH action now has been studied in detail in blue damselfish (*Chrysiptera cyanea*) melanophores (Oshima *et al.,* 1985).

Pigment aggregation in chromatophores is controlled primarily by sympathetic postganglionic fibers and peripheral transmission is adrenergic in many teleosts (Fujii, 1961; Fujii and Miyashita, 1975; *cf.* review by Fujii and Oshima, 1986). Recently, however, unusual melanophores have been reported in some species of siluroid catfish which receive cholinergic innervation (Fujii and Miyashita, 1976; Fujii *et al.,* 1982; Kasukawa *et al.,* 1986). Furthermore, melanophores which possess both alpha adrenoceptors and muscarinic cholinoceptors have been found in mailed cat-

Received 28 March 1986; accepted 7 July 1986.
* Author to whom correspondence should be addressed.

fish belonging to the genus *Corydoras* (Callichthyidae) (Kasukawa and Fujii, 1985; Kasukawa *et al.,* 1986). The action of the peptide on those cells as yet has not been studied. The present paper examines comparatively the effect of the hormone on melanophores of several teleost species in an attempt to determine whether MCH is a general color change hormone in Teleostei.

Materials and Methods

The topmouth gudgeon (*Pseudorasbora parva*), rose bitterling (*Rhodeus ocellatus ocellatus*), and the top minnow (*Gambusia affinis*) were collected from Lake Inba, Chiba Prefecture. The marine catfish (*Plotosus lineatus*), rudder fish (*Girella punctata*), footballer (*Microcanthus strigatus*), five-banded damselfish (*Abudefduf vaigiensis*), dusky damsel (*A. notatus*), multicolorfin rainbowfish (*Halichoeres poecilopterus*), gluttonous goby (*Chasmichthys gulosus*), frogfish (*Istiblennius enosimae*), redfin velvetfish (*Hypodytes rubripinnis*), scribbled toby (*Canthigaster rivulata*), and the grass puffer (*Takifugu niphobles*) were collected near the Kominato Marine Biological Laboratory, Awa, Chiba Prefecture. Juveniles of the Japanese flounder (*Paralichthys olivaceus*) were supplied by the Chiba Prefectural Seawater Fisheries Experimental Station. Other species were obtained from local retail sources. All species are enumerated without omission later in the Results section. Freshwater and marine materials were maintained in freshwater and seawater aquaria, respectively. All materials were kept there for at least two days before use.

Split fin pieces from either the tail or dorsal fin of some species were prepared according to a method described previously (Fujii, 1959). As for the eel, siluroid catfish, and other scaleless species, small skin specimens from the dorsal trunk were prepared according to the method of Fujii and Miyashita (1976). In other teleosteans, scales were excised from the dorsal trunk. In the peppered catfish, *Corydoras paleatus,* melanophores on the inner surface of an isolated scale were observed (Kasukawa and Fujii, 1985). All the skin specimens were prepared in a physiological solution of the following composition (m*M*): NaCl 128, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.8, D-glucose 5.6, Tris-HCl buffer 5.0 (pH 7.2).

In some experiments, nervous elements around the chromatophores were stimulated chemically to liberate sympathetic pigment-aggregating transmitter (Fujii, 1959). For this purpose, a saline solution containing 50 m*M* K^+ in which the concentration of Na^+ was lowered to maintain constant tonicity was employed (Kasukawa and Fujii, 1984).

Other procedures and experimental arrangements were essentially the same as those described elsewhere (Fujii and Miyashita, 1975). To record the response of melanophores photoelectrically, however, an improved method was adopted (Oshima and Fujii, 1984).

The drugs used were norepinephrine hydrochloride (Sankyo, Tokyo), phentolamine mesylate (Ciba-Geigy, Basel), propranolol hydrochloride (Sigma Chemical, St. Louis), acetylcholine chloride (Daiichi Seiyaku, Tokyo), atropine sulfate (Tanabe Seiyaku, Tokyo), melatonin (Nakarai Chemical, Kyoto), and alpha melanophore-stimulating hormone (alpha-MSH, Sigma Chemical). Synthetic salmon melanin-concentrating hormone (MCH) was provided by Dr. Mac E. Hadley of the University of Arizona.

All experiments were performed at a room temperature between 20 and 26°C.

TABLE I

Teleostei materials employed in the present study to examine the action of MCH on melanophores, which were all proved sensitive to the peptide

ORDER Family	Zoological name	Common name
ANGUILLIFORMES		
Anguillidae	*Anguilla japonica*	Japanese eel
CYPRINIFORMES		
Cyprinidae	*Pseudorasbora parva*	topmouth gudgeon
	Rhodeus ocellatus ocellatus	rose bitterling
	Brachydanio rerio	zebra danio
Cobitidae	*Acanthophthalmus kuhlii*	coolie loach
SILURIFORMES		
Siluridae	*Parasilurus asotus**	Japanese common catfish
	*Kryptopterus bicirrhi**	translucent glass catfish
Ictaluridae	*Ictalurus punctatus*	channel catfish
Plotosidae	*Plotosus lineatus*	marine catfish
Callichthyidae	*Corydoras paleatus***	peppered catfish
CYPRINODONTIFORMES		
Oryziatidae	*Oryzias latipes*	medaka
Poecilidae	*Xiphophorus maculatus*	platyfish (blue variety)
	Gambusia affinis	top minnow
CHANNIFORMES		
Channidae	*Channa argus*	Northern snakehead
PERCIFORMES		
Girellidae	*Girella punctata*	rudder fish
Scorpididae	*Microcanthus strigatus*	footballer
Pomacentridae	*Abudefduf vaigiensis*	five-banded damselfish
	A. notatus	dusky damsel
Labridae	*Halichoeres poecilepterus*	multicolorfin rainbowfish
Gobiidae	*Rhinogobius brunneus*	common freshwater goby
	Chasmichthys gulosus	gluttonous goby
Blenniidae	*Istiblennius enosimae*	frogfish
SCORPAENIFORMES		
Congiopodidae	*Hypodytes rubripinnis*	redfin velvetfish
PLEURONECTIFORMES		
Paralichthyidae	*Paralichthys olivaceus*	Japanese flounder
TETRAODONTIFORMES		
Tetraodontidae	*Canthigaster rivulata*	scribbled toby
	Takifugu niphobles	grass puffer
	Tetradon fluviatilis	green puffer

* Species in which pigment aggregation in response to nervous stimulation is mediated by muscarinic cholinoceptors (Fujii and Miyashita, 1976; Fujii *et al.*, 1982).

** Species in which melanophores are under adrenergic nervous control, but which possess cholinoceptors of unknown significance (Kasukawa and Fujii, 1985).

RESULTS

Effects of MCH on adrenergically innervated melanophores

The effect of MCH on the melanophores of several fish species in which the pigment cells are adrenergically innervated was studied. These are listed in Table I.

Among these species, the following already have been shown to have adrenergically controlled melanophores: the medaka (Watanabe *et al.*, 1962), top minnow (Colley and Hunt, 1974), common freshwater goby (Naitoh *et al.*, 1985), gluttonous

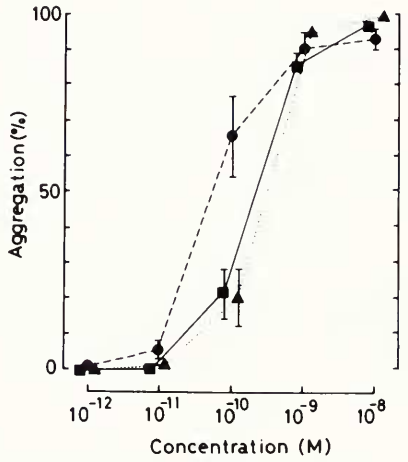

FIGURE 1. Relationships of the concentration of MCH to the magnitude of the pigment-aggregating response of melanophores of the translucent glass catfish, *Kryptopterus bicirrhi* (circles), the Japanese catfish, *Parasilurus asotus* (squares), and the zebra danio, *Brachydanio rerio* (triangles). MCH solutions of various strengths were applied for 7 min. The treatment was immediately followed by a 5-min application with 5×10^{-5} *M* norepinephrine or 10^{-5} *M* melatonin solution to bring about the full level of pigment aggregation in zebra danio or catfish melanophores, respectively. Abscissa: molar concentration of MCH (logarithmic scale). Ordinate: magnitude of response attained during MCH treatment in percentage. Each point is the mean of seven measurements on different animals. Vertical lines indicate SE.

goby (Fujii, 1961), and the Japanese flounder (Nagai *et al.*, 1985). These results were confirmed in the present study. In addition, our present pharmacological tests on other species listed above indicate that their pigment cells were likewise under the control of the adrenergic system. The exceptional cases in which melanophores are controlled cholinergically will be dealt with later.

In all of the aforementioned species, MCH induced a remarkable pigment aggregation in melanophores. After noting this, we made a quantitative measurement of one species, *i.e.*, the zebra danio, *Brachydanio rerio*. As shown in Figure 1, MCH acted on the effector cells dose-dependently, and the minimal effective concentration was estimated to be less than 10^{-10} *M*.

In many other species, too, the peptide was found to be effective at concentrations of not more than 10^{-10} *M*. Strangely, however, the melanophores of all three tetraodontid species examined were found to be less sensitive to MCH: discernible responses were only observable at above 10^{-8} *M*.

In melanophores of many of the above-mentioned species, the pineal hormone, melatonin, has been shown to be very effective in arousing pigment aggregation. Even after withdrawal of the hormone, a moderately aggregated state continued for a long period of time, as exhibited in Figure 2A, in which the response of melanophores of the topmouth gudgeon is recorded. When MCH was added immediately after the melatonin treatment, a further aggregation took place, as shown in Figure 2B. On the other hand, alpha-MSH, used as the control peptide produced a rapid dispersion of cellular inclusions (Fig. 2C). It was thus concluded that MCH has no ability to disperse melanosomes, but is definitely pigment aggregating.

It also should be noted here that the action of melatonin was rather differential among melanophores in many species as has already been reported in some species of fish (Reed, 1968; Fujii and Taguchi, 1969; *cf.*, Fujii and Oshima, 1986). An exam-

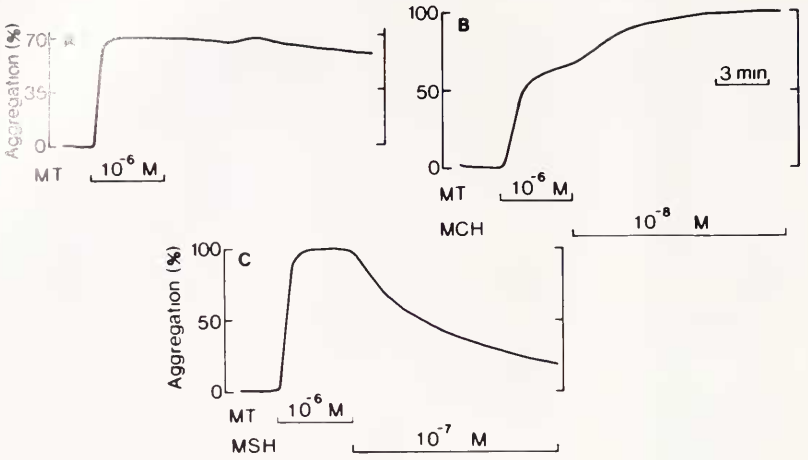

FIGURE 2. A set of recordings of the response of melanophores of the topmouth gudgeon, *Pseudorasbora parva,* indicating that MCH had no pigment-dispersing effect. The recordings were obtained on different scales but from the same fish. The magnitude of response is expressed as the percentage of the maximal level of pigment aggregation. In each recording, the scale was first treated with 10^{-6} *M* melatonin (MT) for 4 min. Then the perfusing medium was changed to physiological saline (A), 10^{-8} *M* MCH (B), or to 10^{-7} *M* MSH (C).

ple of this differential responsiveness to melatonin is exhibited in the melanophores of the topmouth gudgeon (Fig. 3B). On the other hand, the response to MCH proceeded quite simultaneously among the cells (Fig. 3C).

An alpha-adrenolytic agent, phentolamine, had no influence on the melanin-aggregating action of the peptide. As an example of the records, the response of the topmouth gudgeon is exhibited in Figure 4. The melanin-aggregating action of K^+, however, was easily inhibited by phentolamine. This ion has been known to act on chromatic neural elements around the pigment cells to release neurotransmitter (Fujii, 1959). It was concluded, therefore, that MCH acts directly on the target cells and not through activities of the neural elements. Although not shown in the figure, it was further shown that a beta adrenergic blocker, propranolol, had no effect on the action of MCH. These results further indicate that MCH acts independently of alpha and beta adrenoceptors.

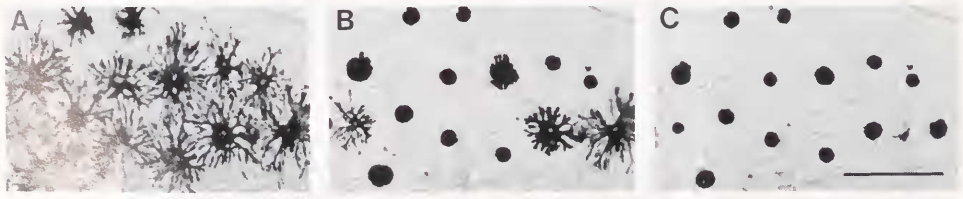

FIGURE 3. Photomicrographs of part of a scale isolated from dorsal part of the topmouth gudgeon, showing the effects of melatonin and MCH. Scale bar indicates 150 μm. A: Equilibrated in physiological saline. Melanosomes in melanophores are in a fully dispersed state. B: 10 min after the application of 5×10^{-7} *M* melatonin. The pigmentary organelles in some melanophores remain in a dispersed state, while in others they have become completely aggregated in the perikarya. C: 10 min after the application of 10^{-7} *M* MCH. Melanosomes in all melanophores are completely aggregated.

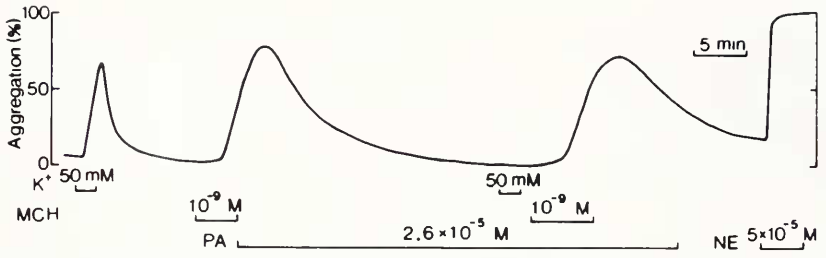

FIGURE 4. Typical recording showing that the alpha adrenolytic agent, phentolamine (PA), did not block the melanin-aggregating effect of MCH in the topmouth gudgeon, *Pseudorasbora parva.* Ordinate: magnitude of response as percentage of the maximal pigment aggregation attained finally by 5×10^{-5} *M* norepinephrine (NE). 50 m*M* K^+ Ringer (K^+) was used to stimulate adrenergic neural elements around the melanophores, and the action was shown to be antagonized by phentolamine (PA).

The melanosome-aggregating response to MCH proceeded quite normally even in the absence of Ca ions. This is in marked contrast to the alpha-MSH action on teleostean chromatophores, in which Ca^{2+} was definitely required (Fujii and Miyashita, 1980; Iga and Takabatake, 1982; Oshima and Fujii, 1985).

Melanophores of the European eel, *Anguilla anguilla,* already have been recognized to be influenced only weakly by the nervous system (Neill, 1940; Gilham and Baker, 1984). Recently, Baker and Rance (1983), however, indicated that their melanophores were responsive to MCH. Although the species used was different, melanophores of the Japanese eel (*A. japonica*) were found in this study to respond quite well to MCH by pigment aggregation.

Effect of MCH on cholinergically innervated melanophores

MCH effectively induced the aggregation of melanophore pigment in the translucent glass catfish, *Kryptopterus bicirrhi,* and the Japanese common siluroid, *Parasilurus asotus,* as seen in Figures 5 and 6, respectively. Both fish belong to the family Siluridae (Siluriformes), and recently have been shown to have melanophores cholinergically controlled (Fujii and Miyashita, 1976; Fujii *et al.,* 1982; *cf.* also Table I). The action of MCH was not inhibited by atropine, a muscarinic cholinolytic, but the pigment-aggregating effect of K^+ was completely blocked by the same drug (Fig. 5). In addition, divalent cation-withdrawal exhibited no influence on the action of MCH (Fig. 6).

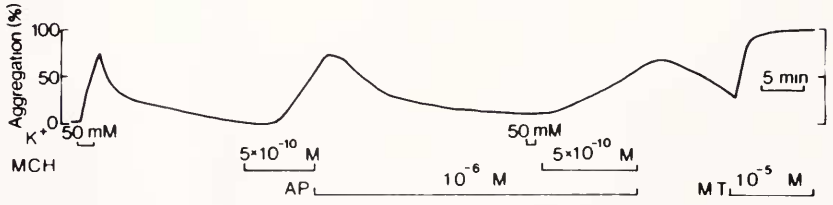

FIGURE 5. Typical recording showing the ineffectiveness of the muscarinic cholinolytic agent, atropine (AP), in blocking the pigment-aggregating action of MCH on melanophores of the translucent glass catfish, *Krytopterus bicirrhi.* Melatonin (MT; 10^{-5} *M*) was applied finally to bring about the full level of melanosome aggregation. 50 m*M* K^+ Ringer (K^+) was used for neural stimulation.

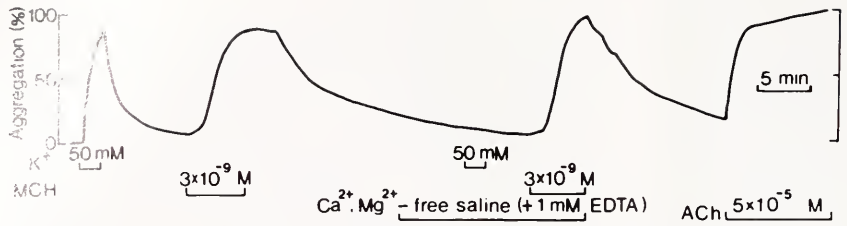

FIGURE 6. Typical recording of the response of Japanese common catfish (*Parasilurus asotus*) melanophores to MCH, showing that the pigment-aggregating effect of the peptide was still remarkable in Ca^{2+}-free saline, in which the release of peripheral stores of neurotransmitter (ACh) was inhibited. 50 m*M* K^+ Ringer (K^+) was used for neural stimulation. Finally, 5×10^{-5} *M* acetylcholine (ACh) was applied to induce the full aggregation of melanosomes.

The relationship between the strength of MCH and the magnitude of the target cell response in the glass catfish and the common siluroid was revealed to be dose-dependent, as shown in Figure 1. In both species, the threshold concentration was estimated to be not more than 10^{-10} *M*, and a response larger than 80% of the maximal one was induceable at a concentration of 10^{-9} *M*.

Effects of MCH on melanophores possessing both alpha-adrenoceptors and cholinoceptors

Melanophores of the mailed catfish of the genus *Corydoras* (Callichthyidae, Siluriformes) possess melanosome-aggregating cholinoceptors of the muscarinic type, notwithstanding the fact that they are also under the control of adrenergic fibers (Kasukawa and Fujii, 1985; Kasukawa *et al.,* 1986; *cf.* also Table I). Employing a representative species from this genus, *i.e.,* the peppered catfish (*C. paleatus*), we found that MCH had the same potent action to aggregate melanophore inclusions as it did in other teleosteans. Neither adrenolytic nor muscarinic cholinolytic agents interfered with the MCH effect. Furthermore, Ca^{2+}-withdrawal did not affect the responsiveness to the hormone.

DISCUSSION

Using a number of teleost species from a variety of families and orders, we have demonstrated in the present study that MCH is very effective in bringing about a notable pigment aggregation not only in adrenergically but also in cholinergically innervated melanophores, and further that the mode of action of the peptide is the very same among those pigment cells. Its minimum effective concentration was estimated to be less than 10^{-10} *M* in most teleosts, with the exception of three swellfish: the scribbled toby, the grass puffer, and the green puffer.

Recent quantitative studies on the effects of other pigment-motor hormonal substances, *i.e.,* MSH (Fujii and Miyashita, 1982) and melatonin (Fujii and Miyashita, 1978), showed that the threshold concentration was about 10^{-10} *M*. Our present results on MCH agree well with those data. In the above-mentioned swellfish, however, the threshold concentration of MCH for inducing a discernible melanin aggregation was estimated to have an exceptionally high value of about 10^{-8} *M*. Further comparative studies among species of Tetraodontidae are thus required to give an adequate explanation for such lower sensitivity to the hormone.

Using purified or synthetic chum salmon MCH, Kawauchi *et al.* (1983), Wilkes

et al. (1984), and Oshima *et al.* (1985) have already demonstrated MCH's pigment-aggregating action on the adrenergically innervated melanophores of a number of teleost species. These species are the rainbow trout, *Salmo gairdneri* (Salmonidae, Salmoniformes), the carp, *Cyprinus carpio* (Cyprinidae, Cypriniformes), the crucian carp, *Carassius auratus* (Cyprinidae, Cypriniformes), the fathead minnow, *Pimephales promelas* (Cyprinidae, Cypriniformes), the guppy, *Lebistes reticulatus* (Poeciliidae, Cyprinodontiformes), the swordtail, *Xiphophorus helleri* (Poeciliidae, Cyprinodontiformes), the black rockfish, *Sebastes schlegeli* (Scorpaenidae, Scorpaeniformes), the fat greenling, *Hexagrommos otakii* (Hexagrammidae, Scorpaeniformes), the tilapia, *Sarotherodon mossambicus* (Cichlidae, Perciformes), the blue damselfish, *Chrysiptera cyanea* (Pomacentridae, Perciformes), and the clingfish, *Gobiesox pinniger* (Gobiesocidae, Gobiesociformes). Incidentally, Wilkes *et al.* (1984) referred to the last fish as *Gobeisox,* giving neither the common name nor any taxonomical keys. Apparently the genus name given involved a clerical error. It should have read *Gobiesox,* the genus, we confirmed, comprising the species *G. pinniger* which is indigenous to the United States. In addition, Baker and her colleagues (Baker and Rance, 1983; Gilham and Baker, 1984) have also shown that partially purified MCH of trout (*Salmo gairdneri*) origin caused pigment aggregation in melanophores of the European eel, *Anguilla anguilla.*

In addition to the above-mentioned species, we have surveyed 27 other species of fish in this study, and found that each species' melanophores were reactive to MCH by pigment aggregation. To date, we have not encountered any fish in which the melanophores have been unresponsive to this hormone. From these results it may be deduced that MCH is a physiologically functioning hormone throughout the teleost class. As suggested by some workers (Enami, 1955; Baker and Rance, 1983; Naito *et al.,* 1985), the peptide, synthesized in the hypothalamus, may be transferred to the pituitary, wherefrom it is secreted as a hormone to blanch the integument in most teleosts.

The mode of action of MCH on cholinergically controlled melanophores has been studied for the first time in this work. Pharmacological analyses clearly indicate that the melanosome-aggregating action of MCH is not inhibited by a muscarinic cholinolytic agent, atropine, which normally interferes with peripheral transmission to the effector cells.

It is now known that Ca^{2+} is required for the release of neurotransmitters from chromatic nerves of either adrenergic (Fujii and Novales, 1972; Takabatake and Iga, 1982) or cholinergic type (Kasukawa and Fujii, 1985). Therefore, we have tried to test the influence of MCH in the absence of this ion. The result was that MCH exhibited its potent action irrespective of the presence of Ca^{2+}. Incidentally, we have lately reached the same conclusion on adrenergically controlled melanophores (Oshima *et al.,* 1985). In reconfirming that conclusion, we found that in cholinergically innervated melanophores the action of MCH is mediated through the specific receptors for MCH.

During the same period of time, it was noted that the differential responsiveness to melatonin seen among the melanophores of individual animals or on isolated pieces of skin may be responsible for the formation of the characteristic color pattern seen in the pencil fish *Nannostomus* (Reed, 1968), and possibly in some other teleosts (Fujii and Taguchi, 1969; Fujii and Miyashita, 1978; *cf.* also review by Fujii and Oshima, 1986). During the present investigation, we also found that the responsiveness to melatonin was actually very differential among melanophores distributing, for instance, over an isolated scale of the topmouth gudgeon. In contrast, MCH induced much more even responses among cells in most fish species. Therefore, we

assume that MCH serves as a color change hormone whose action counteracts that of alpha-MSH. In preliminary experiments on the zebra danio, topmouth gudgeon, and the glass catfish, we examined the response of melanophores to solutions containing both alpha-MSH and MCH. When the strengths of both hormones were approximately equal, a moderately aggregated state of pigment could be maintained in the melanophores. It is still uncertain, however, to just what extent MCH takes part in actual physiological color changes in teleosts. For a full understanding of the role of the peptide, quantitative analyses, such as correlated measurement by radioimmunoassay of plasma MCH levels and reliable assessment of integumental chromatic reactions *in vivo,* should be performed.

The individual membranes surrounding teleost melanophores are endowed with a number of ligand receptor species which mediate pigment movements within the cell (Fujii and Oshima, 1986). They include at least those for MSH (Fujii and Miyashita, 1982), melatonin (Fujii and Miyashita, 1978), alpha- (Fujii and Miyashita, 1975), and beta-adrenergic amines (Miyashita and Fujii, 1975), and for adenosine (Miyashita *et al.,* 1984). The receptors for MCH now may be added to this list. Although not all of the above-mentioned receptors are always present in a single melanophore of a certain species of animal, very frequently many of them do exist, each carrying out its individual function (Fujii and Oshima, 1986). We know of no other instance among effector cells in which so many kinds of receptors exist and function to regulate the cells' motile responses. Representing a remarkable instance of possession of so many kinds of receptors, therefore, the fish melanophore may prove useful in studying the mechanisms by which many or large numbers of input signals are integrated into a simple vectoral movement of pigmentary organelles.

That so many receptor sorts are at work seems to signify the necessity for subtle and delicate regulation of the chromatophores for animals living in their habitat. If we remind ourselves of the fact that the color changes and color patterns displayed by animals are very important in their strategies for survival, the situation can even more easily be understood.

We hope that our present comparative studies at the cellular level will be of some use not only for further understanding of the cellular mechanism of hormonal action, but also for comprehension of the above-mentioned organismal phenomena of both ethological and ecological interests.

Acknowledgments

We are grateful to Professor M. E. Hadley of the University of Arizona for his kind gift of synthetic salmon MCH. Our thanks are also due to Dr. R. Arai, Department of Zoology, National Science Museum, Tokyo, for providing current information about the classification of some materials, and to the staff of the Chiba Prefectural Seawater Fisheries Experimental Station for the supply of flounders. Many other marine species were collected near the Kominato Marine Biological Laboratory, Faculty of Science, Chiba University, Awa, Chiba Prefecture. On this occasion, we would like to express our appreciation to the staff there for offering us the facilities concerned. This work was partly supported by grants from the Ministry of Education, Science and Culture of Japan.

LITERATURE CITED

Baker, B. I., and T. A. Rance. 1983. Further observations on the distribution and properties of teleost melanin concentrating hormone. *Gen. Comp. Endocrinol.* **50:** 423–431.

COLLEY, A. M., AND E. L. HUNT. 1974. Cyclic AMP and adrenergic receptors in *Gambusia affinis* melanophore response *in vitro*. *Gen. Comp. Endocrinol.* **23:** 154–163.

ENAMI, M. 1955. Melanophore-concentrating hormone (MCH) of possible hypothalamic origin in the catfish *Parasilurus*. *Science* **121:** 36–37.

FUJII, R. 1959. Mechanism of ionic action in the melanophore system of fish—I. Melanophore-concentrating action of potassium and some other ions. *Annot. Zool. Japn.* **32:** 47–58.

FUJII, R. 1961. Demonstration of the adrenergic nature of transmission at the junction between melanophore-concentrating nerve and melanophore in bony fish. *J. Fac. Sci. Univ. Tokyo* Sect. IV **9:** 171–196.

FUJII, R., AND Y. MIYASHITA. 1975. Receptor mechanisms in fish chromatophores—I. Alpha nature of adrenoceptors mediating melanosome aggregation in guppy melanophores. *Comp. Biochem. Physiol.* **51C:** 171–178.

FUJII, R., AND Y. MIYASHITA. 1976. Receptor mechanisms in fish chromatophores—III. Neurally controlled melanosome aggregation in a siluroid (*Parasilurus asotus*) is strangely mediated by cholinoceptors. *Comp. Biochem. Physiol.* **55C:** 43–49.

FUJII, R., AND Y. MIYASHITA. 1978. Receptor mechanisms in fish chromatophores—IV. Effects of melatonin and related substances on dermal and epidermal melanophores of the siluroid, *Parasilurus asotus*. *Comp. Biochem. Physiol.* **59C:** 59–63.

FUJII, R., AND Y. MIYASHITA. 1980. Action of melanophore-stimulating hormone on dermal and epidermal melanophores of the siluroid, *Parasilurus asotus*. *Yale J. Biol. Med.* **53:** 422.

FUJII, R., AND Y. MIYASHITA. 1982. Receptor mechanisms in fish chromatophores—V. MSH disperses melanosomes in both dermal and epidermal melanophores of a catfish (*Parasilurus asotus*). *Comp. Biochem. Physiol.* **71C:** 1–6.

FUJII, R., AND R. R. NOVALES. 1972. Nervous control of melanosome movements in vertebrate melanophores. Pp. 315–326 in *Pigmentation: Its Genesis and Biologic Control*, V. Riley, ed. Appleton-Century-Crofts, New York.

FUJII, R., AND S. TAGUCHI. 1969. The responses of fish melanophores to some melanin-aggregating and dispersing agents in potassium-rich medium. *Annot. Zool. Japn.* **42:** 176–182.

FUJII, R., Y. MIYASHITA, AND Y. FUJII. 1982. Muscarinic cholinoceptors mediate neurally evoked pigment aggregation in glass catfish melanophores. *J. Neural Trans.* **54:** 29–39.

FUJII R., AND N. OSHIMA. 1986. Control of chromatophore movements in teleost fishes. *Zool. Sci.* **3:** 13–47.

GILHAM, I. D., AND B. I. BAKER. 1984. Evidence for the participation of a melanin-concentrating hormone in physiological color change in the eel. *J. Endocrinol.* **102:** 237–243.

IGA, T., AND I. TAKABATAKE. 1982. Action of melanophore-stimulating hormone on melanophores of the cyprinid fish *Zacco temmincki*. *Comp. Biochem. Physiol.* **73C:** 51–55.

IMAI, K. 1958. Extraction of melanophore concentrating hormone (MCH) from the pituitary of fishes. *Endocrinol. Japn.* **5:** 34–48.

KASUKAWA, H., AND R. FUJII. 1984. Potassium ions act to release transmitter from "cholinergic" sympathetic postganglionic fiber to the glass catfish melanophore. *Zool. Sci.* **1:** 553–559.

KASUKAWA, H., AND R. FUJII. 1985. Receptor mechanisms in fish chromatophores—VII. Muscarinic cholinoceptors and alpha adrenoceptors, both mediating pigment aggregation, strangely coexist in *Corydoras* melanophores. *Comp. Biochem. Physiol.* **80C:** 211–215.

KASUKAWA, H., N. OSHIMA, AND R. FUJII. 1986. A comparative survey of the type of sympathetic neuromelanophore transmission in catfishes. *Comp. Biochem. Physiol.* (in press).

KAWAUCHI, H., I. KAWAZOE, M. TSUBOKAWA, M. KISHIDA, AND B. I. BAKER 1983. Characterization of melanin-concentrating hormone in chum salmon piuitaries. *Nature* **305:** 321–323.

MIYASHITA, Y., AND R. FUJII. 1975. Receptor mechanisms in fish chromatophores—II. Evidence for beta adrenoceptors mediating melanosome dispersion in guppy melanophores. *Comp. Biochem. Physiol.* **51C:** 179–187.

MIYASHITA, Y., T. KUMAZAWA, AND R. FUJII. 1984. Receptor mechanisms in fish chromatophores—VI. Adenosine receptors mediate pigment aggregation in guppy and catfish melanophores. *Comp. Biochem. Physiol.* **77C:** 205–210.

NAITO, N., Y. NAKAI, H. KAWAUCHI, AND Y. HAYASHI. 1985. Immunocytochemical identification of melanin-concentrating hormone in the brain and pituitary gland of the teleost fishes *Oncorhynchus keta* and *Salmo gairdneri*. *Cell Tissue Res.* **242:** 41–48.

NAITOH, T., A. MORIKAWA, AND Y. OMURA. 1985. Adaptation of a common freshwater goby, yoshinobori, *Rhinogobius brunneus* Temminck et Schlegel to various backgrounds including those containing different sizes of black and white checkerboard squares. *Zool. Sci.* **2:** 59–63.

NAGAI, M., H. KASUKAWA, AND R. FUJII. 1985. Fundamental studies on mottled albinism occurring among flounders in artificial proliferation. *Zool. Sci.* **3:** 885.

NEILL, R. M. 1940. On the existence of two types of chromatic behaviour in teleostean fishes. *J. Exp. Biol.* **17:** 74–98.

OSHIMA, N., AND R. FUJII. 1984. A precision photoelectric method for recording chromatophore response *in vitro*. *Zool. Sci.* **1:** 553–559.

OSHIMA, N., AND R. FUJII. 1985. Calcium requirement for MSH action on non-melanophoral chromatophores of some teleosts. *Zool. Sci.* **2:** 127–129.

OSHIMA, N., H. KASUKAWA, R. FUJII, B. C. WILKES, N. J. HRUBY, A. M. DE L. CASRUCCI, AND M. E. HADLEY. 1985. Melanin concentrating hormone (MCH) effects on teleost (*Chrysiptera cyanea*) melanophores. *J. Exp. Zool.* **234:** 175–180.

REED, B. L. 1968. The control of circadian pigment changes in the pencil fish: a proposed role for melatonin. *Life Sci.* **7,** Pt. II: 961–973.

TAKABATAKE, I., AND T. IGA. 1982. Some properties of pigment-aggregating nerve to fish melanophores. *Zool. Mag. (Tokyo)* **91:** 429.

WATANABE, M., M. KOBAYASHI, AND K. S. IWATA. 1962. The action of certain autonomic drugs on the fish melanophore. *Biol. J. Okayama Univ.* **8:** 103–114.

WILKES, B. C., V. J. HRUBY, A. M. DE L. CASTRUCCI, W. C. SHERBROOKE, AND MAC E. HADLEY. 1984. Synthesis of a cyclic melanotropic peptide exhibiting both melanin-concentrating and dispersing activities. *Science* **224:** 1111–1113.